

Clean Copy of Amended Claims

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1 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

2 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

3 (amended). The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

4 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;

(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

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5 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

6 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

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9 (amended). The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

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12 (amended). The method of claim 1, wherein said selection is performed by culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

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15 (amended). The method of claim 14, wherein said layer is a layer of liquid medium.

16 (amended). The method of claim 14, wherein said layer is a layer of gelled medium.

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19 (amended). The method of claim 1 which further comprises the eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

20 (amended). The method of claim 19, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

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21 (amended). The method of claim 20, wherein said layer is a layer of liquid medium.

22 (amended). The method of claim 20, wherein said layer is a layer of gelled medium.

25 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

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incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

26 (amended). The method of claim 25, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

27 (amended). The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

28 (amended). The method of claim 26, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and
selecting transformed cells.

29 (amended). The method of claim 26, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

30 (amended). The method of claim 28, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

31 (amended). The method of claim 25, wherein said damage to cells is minimized by:
(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and
(c) recovering washed cells with minimal damage.

32 (amended). The method of claim 31, wherein pine cells are plated onto a support
membrane prior to *Agrobacterium* transformation.

33 (amended). The method of claim 31, wherein pine cells are plated onto a support
membrane subsequent to *Agrobacterium* transformation.

34 (amended). The method of claim 31, wherein said selection is performed by
culturing cells which have been incubated with *Agrobacterium* on a support membrane
placed over a gel medium;

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contacting said cells with a selection agent; and
selecting transformed cells.

35 (amended). The method of claim 31, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

36 (amended). The method of claim 34, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

37 (amended). The method of claim 25, wherein said selection is performed by
culturing cells which have been incubated with *Agrobacterium* on a support membrane
placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

38 (amended). The method of claim 25, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

39 (amended). A method for minimizing damage to transformed cells of pine of the genus
Pinus subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation
which comprises:

(a) washing transformed cells of the subgenus *Pinus* in a liquid wash medium;

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- (b) plating said cells on a support membrane;
 - (c) suspending said cells in a liquid wash medium; and
 - (d) recovering washed cells with minimal physical damage.
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43 (amended). The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

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46 (amended). A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

47 (amended). The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents, said layer is positioned on said gel medium.

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49 (amended). The method of claim 47, wherein said layer is a layer of liquid medium.

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52 (amended). A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

- culturing cells of the *Pinus* subgenus subsequent to transformation on a support membrane placed over a gel medium;
- contacting said cells with a selection agent; and
- selecting transformed cells.

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55 (amended). The method of claim 54, wherein said layer is a layer of liquid medium.

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58 (amended). A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an
eradicant, said layer positioned in or over a gel medium; and
recovering cells from which said *Agrobacterium* contaminant has been eradicated.

59 (amended). The method of claim 58, wherein said layer is a layer of liquid medium.

60 (amended). The method of claim 58, wherein said layer is a layer of gelled medium.

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